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Application Note

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Sartobind® Rapid A as an Efficient, Cost-Effective Alternative to Traditional Protein A Resin Chromatography for Smaller-Scale mAb Production

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Abstract

Protein A resin-based chromatography is the current industry standard for purifying monoclonal antibodies and Fc-based fusion proteins. However, it represents the most rate-limiting and costly step of the downstream purification process because Protein A chromatography resins are restricted in terms of flow rate and, subsequently, productivity. As a result, large upfront investments are required, and the full lifespan of the resin cannot be fully utilized.

There is significant interest in improving this step to reduce the cost of goods and increase the productivity of the down-stream process. Sartobind® Rapid A membranes enable the operation of capture chromatography without long set-up and process times for affinity chromatography, or the need for highly specialized expertise.

This application note is divided into two parts. First, it presents the results of a study by our partners at Nanogen on the efficiency and impurity removal performance of Sartobind® Rapid A membranes versus traditional resin-based processes. Second, it evaluates the financial impact of converting a Protein A resin-based process to Sartobind® Rapid based on a cost analysis.

Introduction

Therapeutic monoclonal antibodies (mAbs) have a substantial presence in the current biopharmaceutical industry. mAbs produced by mammalian cell culture possess an Fc-region and are typically purified by affinity chromatography with Protein A resins. This is a critical but expensive step, representing a key opportunity for optimization.

Multiple approaches have been developed to overcome and reduce the cost of affinity capture, such as using lower resin volumes and performing multiple cycles within a batch to utilize the resin for its entire lifetime (150–200 cycles). Some recombinant Protein A chromatography media with higher dynamic binding capacity (DBC) and better compatibility with cleaning solutions like sodium hydroxide (NaOH) are now available on the market, creating more efficient purification processes.

Sartorius, a leading supplier of membrane chromatography devices, has developed a new agarose-based membrane platform, Sartobind® Rapid. The first commercially available modification of this platform, Sartobind® Rapid A, uses a Protein A ligand. Sartobind® Rapid membranes have large pores (> 4 µm) but have a thin diffusive agarose gel-layer, resulting in similar binding capacities even at lower residence times. This gives Sartobind® Rapid A the unique ability to support faster flow rates and perform multiple cycles within one or more batches (fully utilizing the entire lifespan of the device). As such, implementing the Sartobind® Rapid A adds significant value to mAb purification processes and helps reduce the overall cost of mAb production.

Nanogen is a biopharmaceutical manufacturing organization based in Ho Chi Minh City, Vietnam. One of their objectives was to increase the efficiency of their resin-based mAb capture step with novel chromatography technologies that can deliver similar or better impurity removal and product recovery. Nanogen previously implemented other Sartobind® membranes in their manufacturing facilities for polishing steps and wanted to test the performance of Sartobind® Rapid A for the capture step.

Materials and Methods

Equipment Used in This Study

- Sartobind® Rapid A 1.2 mL Nano
- Sartopore® 2 filter
- ÄKTA avant™ chromatography system
- Clarified cell culture harvest
- Buffers (see results section)
- Agilent 1260 Infinity
- Agilent Bio SEC-5 500 µm, 300 Å, 7.8 × 300 mm
- Tosoh TSKGel® Protein A-5PW, 20 µm, 4.6 mm ID × 3.5 cm

Process Configuration

Figure 1 shows an example of the expected chromatograms (UV and conductivity) for the method chosen for the purification of mAbs, as described in detail in Table 1.

Table 1: *Sartobind® Rapid A Purification Conditions*

Step	Buffer	Flowrate [MV/min]	Volume [MV]
Cleaning	100 mM NaOH	5	15
Equilibration	25 mM Tris 7.5 + 0.15 M NaCl		
Loading sample	n.a.	5	38.18 mL/cycle
Re-equilibration	25 mM Tris 7.5 + 0.15 M NaCl	5	15
Washing	25 mM Tris 7.5 + 1 M NaCl		
Citrate exchange	50 mM pH 6.0		
Elution	50 mM pH 3.5		
Quencher	Glycine buffer, 200 g/L, pH 8.5		

Note. This is a standard protocol used for the screening study and has scope for optimizing further to reduce cycle time and buffer consumption

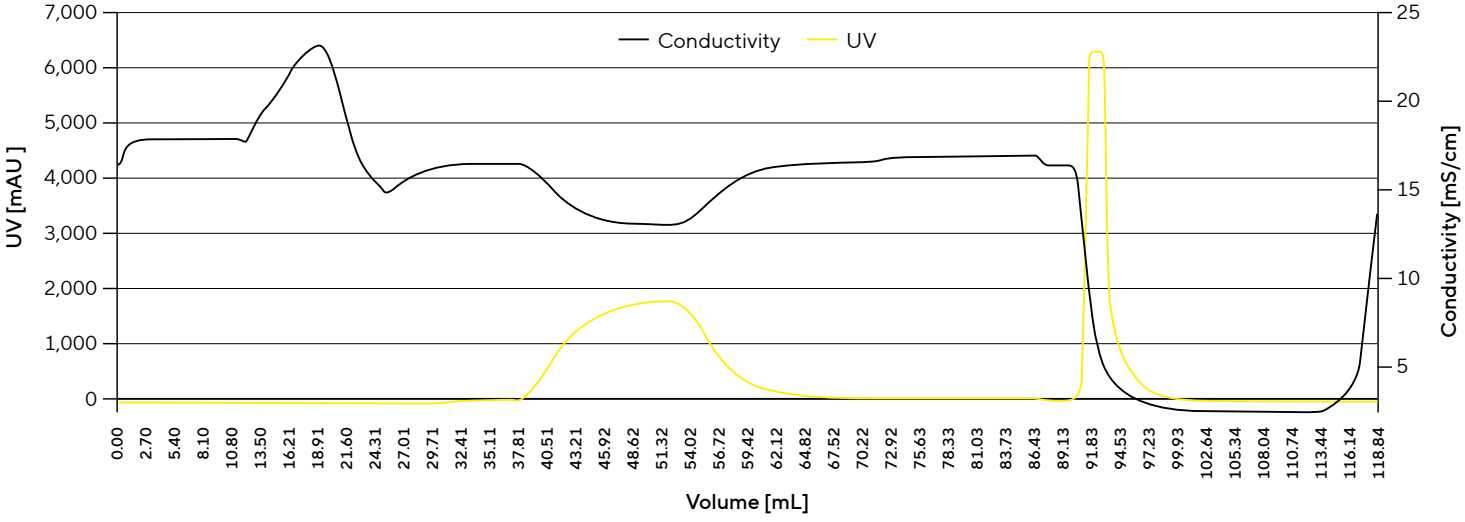
Dynamic Binding Capacity

DBC is a measurement of the actual binding capacity of the resin or membrane at a specific residence time and actual process conditions. A small volume of resin is packed and tested for mAb binding with a defined residence time, and smaller fractions are collected and tested further for traces of loaded mAb on the column. Fractions showing a presence of mAb with a certain concentration are collected to define the DBC at the corresponding percentage of the mAb titer in the post-depth filter harvest*, e.g., DBC at a 10% breakthrough value. Later, for purification runs, a margin of 10–20% is subtracted from the volume of harvest corresponding to the DBC, meaning only 80–90% of the actual binding capacity is used as the operating binding capacity. This avoids mAb loss in the flowthrough fraction due to variation in the harvested material to be loaded. A similar approach was used to measure the DBC of Sartobind® Rapid A at Nanogen.

*Hereafter, 'titer' refers the post-depth filter harvest titer

Analytical Methods
Host cell protein (HCP) was measured by ELISA, and host cell DNA (hcDNA) removal was measured by real-time PCR. The percentages of high-molecular-weight species (HMWS) and low-molecular-weight species (LMWS) were determined by high-performance liquid chromatography (HPLC). The concentration of mAbs was determined by Protein A size-exclusion (SEC)-HPLC.

Figure 1: *Chromatogram of Nanogen's Sartobind® Rapid A Purification Process*



Results and Discussion

Part 1 — Purification

Nanogen investigated on detail the performance of Sartobind® Rapid A in the purification of two mAbs, Trastuzumab (1.01 g/L titer) and Bevacizumab (2.43 g/L titer).

Dynamic Binding Capacity
Sartobind® Rapid A membranes were loaded at four residence times: 12, 24, 60, and 120 seconds, representing flow rates of 5, 2.5, 1, and 0.5 membrane volumes per minute (MV/min). Table 2 shows the resulting DBC at 2% and 10% breakthrough for both mAbs.

Table 2: *Dynamic Binding Capacity of the Sartobind® Rapid A*

Trastuzumab				Bevacizumab			
Flowrate [MV/min]	Residence Time [seconds]	DBC2% [mL]	DBC10% [mL]	Flowrate [MV/min]	Residence Time [seconds]	DBC 2% [mL]	DBC 10% [mL]
5	12	43.00	46.46	5	12	43.50	47.72
2.5	24	49.68	50.60	2.5	24	52.02	58.10
1	60	51.82	52.86	1	60	57.20	59.90
0.5	120	52.68	55.73	0.5	120	61.72	64.02

Table 3: *Impurity Removal Capabilities of the Sartobind® Rapid A After 1 and 150 Cycles Continuously*

		Trastuzumab				Bevacizumab			
Sample	**Cycle Number**	**HCP [%]**	**hcDNA [pg/mg]**	**LMWs [%]**	**HMWS [%]**	**HCP [%]**	**hcDNA [pg/mg]**	**LMWs [%]**	**HMWS [%]**
Cycle 2 Elution	2	<0.1%	<6	0	4.23	0.14	30.91	0	5.18
Cycle 100 Elution	100	<0.1%	<6	0	4.12	0.12	20.03	0	5.09

Even at a flow rate of 5 MV/min, the Sartobind® Rapid A maintains a binding capacity comparable to that of Protein A resins (Table 2). Sartobind® Rapid A benefits from short cycle times completing its cycle in just 15 – 20 minutes at this flow rate, while the equivalent process for resins would take 3 hours. Therefore, the Sartobind® Rapid A can alleviate downstream processing bottlenecks related to processing times and productivity.

Impurity Removal

Nanogen also sought to determine whether the Sartobind® Rapid A could maintain impurity removal efficiency for up to 150 cycles. Table 3 shows the impurities remaining in the eluate of Sartobind® Rapid A-purified samples. For mAbs, the impurity removal capability at cycle 1 is similar to cycle 150, indicating that the Sartobind® Rapid A maintains robust performance across repeated cycles.

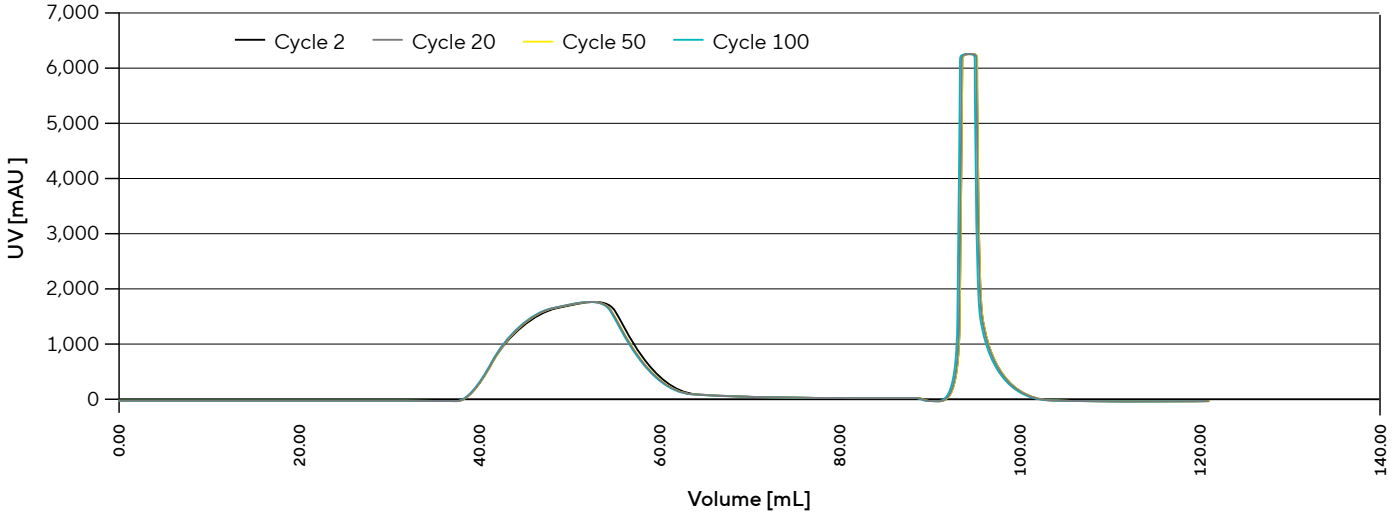
The Sartobind® Rapid A membrane demonstrated binding capacity and impurity removal consistent with Protein A resins for both biomolecules.

Repeatability of Results

The protocol shown in Table 3 was repeated to at least 100 cycles. The graph is obtained from trial results using Trastuzumab as an example.

Figure 1 shows cycle 2, where the graph shows overlaying of the UV and conductivity signals. Figure 2 shows the UV overlay of the Sartobind® Rapid A UV chromatograms from cycles 2, 20, 50, and 100, showing that Sartobind® Rapid A delivers a high degree of repeatability, consistent with the results displayed in Table 3. Cycle 1 is not shown in this application note because Nanogen used cycle 1 as a benchmark on how to operate the Sartobind® Rapid A.

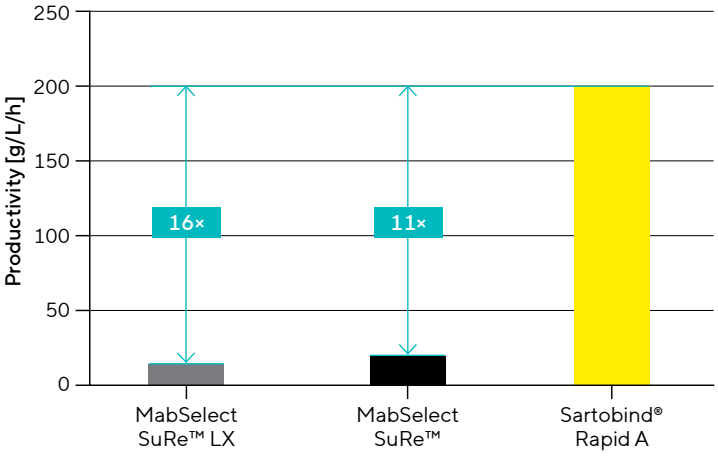
Figure 2: *Chromatogram Overlay for Cycles 2, 20, 50, and 100*



Productivity

Nanogen then compared the productivity of the Sartobind® Rapid A to Protein A resins. MabSelect SuRe™ has a maximum DBC of 25 g/L resin binding capacity (at 6 – 8 minutes residence time), resulting in a productivity of 12 g/L/hour. MabSelect SuRe™ LX has a maximum DBC of 55 g/L resin, resulting in 18.3 g/L/hour productivity. In contrast, the Sartobind® Rapid A offered 200 g/L/hour, an 11- and 16-fold difference compared to MabSelect SuRe™ and MabSelect SuRe™ LX, respectively (Figure 3).

Figure 3: *Productivity of Protein A Resins Compared to Sartobind® Rapid A Membranes*



Part 2 — Cost Analysis

Based on the similar purification performance and higher achievable productivity, Sartobind® Rapid A can therefore drive significant cost savings. MabSelect SuRe™ and MabSelect SuRe™ LX cost around $18,000/L and $27,000/L, respectively, while Sartobind® Rapid A costs approximately $27,000/L. Despite the higher-end list price per volume of membrane, the productivity savings combined with the lower absolute bed-volume required to process the same amount of harvest makes Sartobind® Rapid A more cost-effective.

Figure 4 illustrates the economical benefits of Nanogen's capture process after converting their technology from resin-based to Sartobind® Rapid A. This applies to various molecules produced by Nanogen are presented, along with their expected titer and production rate in Table 4, assuming an average binding capacity for MabSelect SuRe™, MabSelect SuRe™ LX, and Sartobind® Rapid A and lower than experimentally observed DBC for Sartobind® Rapid A (worst-case).

We modeled the cost of production to compare MabSelect SuRe™ and MabSelect SuRe™ LX to Sartobind® Rapid A chromatography with scenarios 1 – 5 shown in Table 5 with the following assumptions:

Model Assumptions

- All hardware depreciated over a seven-year period.
- Buffer costs are estimated to be 3 €/L.
- Comparative recovery of both resin and Sartobind® Rapid A and the cost of the mAb product yield are not included
- The cost of column maintenance (replacement of parts) is not included
- An additional 15% of resins is required during column packing
- The cost of resin is included
- For 200 L production, 200 mL capsules will be used.
- For 500 and 1,000 L production, 800 mL cassette format will be used

Table 5: *Overview of Scenarios for Cost of Goods Calculations*

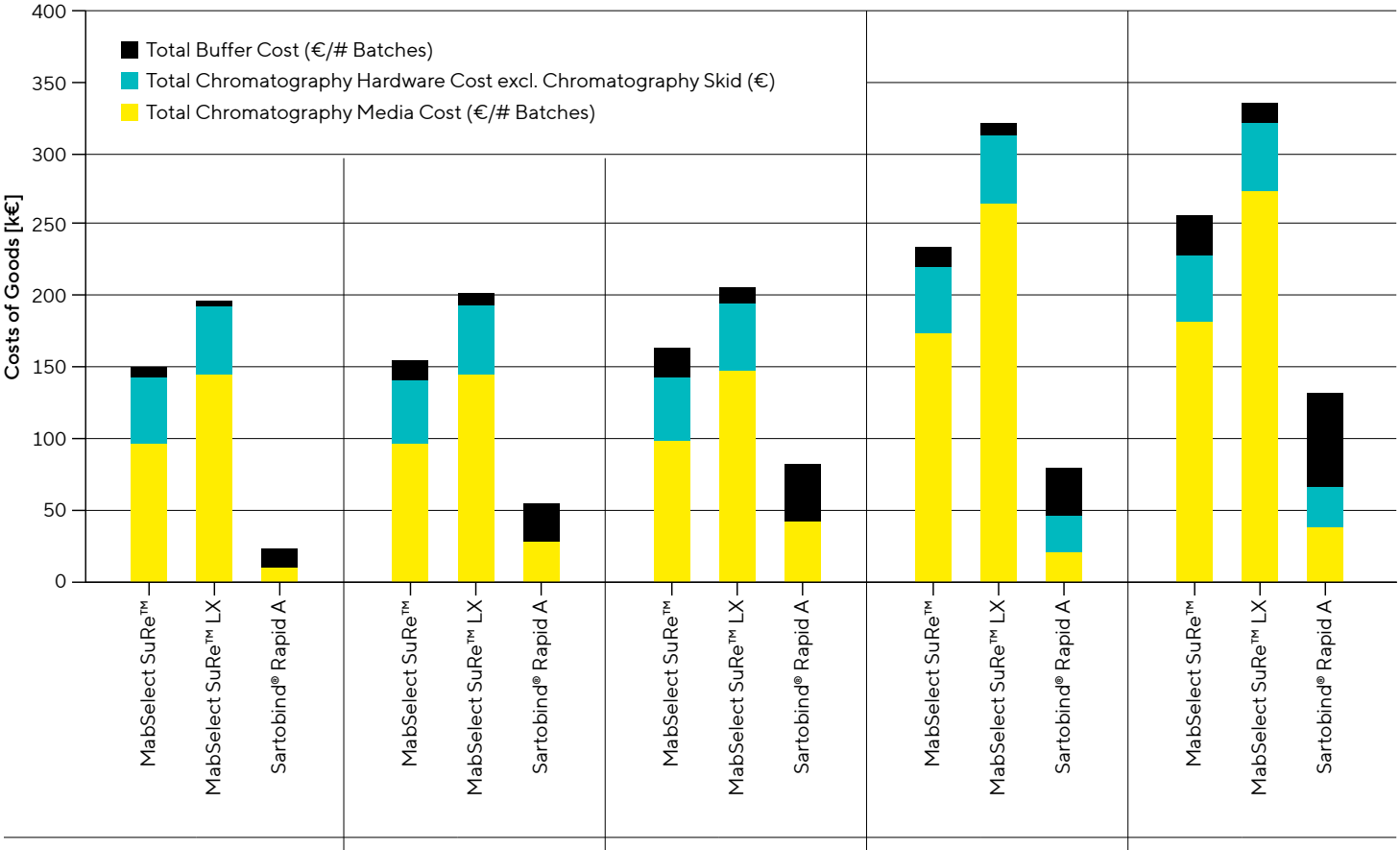
Scenario	Volume [L]	Number of Batches
1	200	4
2	200	8
3	200	12
4	500	4
5	1,000	4

Table 4: *Example of Expected Production Forecast for an Extract of Nanogen's Molecule Pipeline*

Molecule Name	Scale [L]	Post-Depth Filter Harvest Titer [g/L]	Production Rate [# batches/year]	MabSelect SuRe™ avg. DBC 10% [g/L]	MabSelect SuRe™ LX avg. DBC 10% [g/L]	Sartobind® Rapid A avg. DBC 10% [g/L]
Adalimumab	200	3.0	1 – 2	25	55	40
Bevacizumab	200	2 – 2.5	2 – 3	25	55	40
Rituximab	200	2.5	2 – 3	25	55	40
Trastuzumab	1,000	2.5	6	25	55	40

The expected cost of goods (COGs) for Nanogen upon switching to Sartobind® Rapid A are shown in Figure 4.

Figure 4: *Cost of Goods Breakdown: Impact of Batch Volume and Number of Batches per Year, Considering Sartobind® Rapid A and Resin-Based Chromatography Media*



Scenario	1	2	3	4	5
Harvest [L]	200	200	200	500	1,000
Batches Production [#]	4	8	12	4	4

Across all scenarios, Sartobind® Rapid A drove significant cost savings compared to the two resin alternatives. In the case of a high production rate (more than ten batches per year), users can adjust the process times accordingly by using different-sized Sartobind® Rapid A capsules or more modules of the cassette format, rather than being restricted to a single column diameter size. Having more than one column diameter size poses inflexible manufacturing operations, such as the need to maintain multiple columns at a given time. For instance, a user with a 60 cm diameter column can only pack 56 L of 20 cm height resin at a given point. The total processing time for this process is fixed by the diameter of the column. On the other hand, a Sartobind® Rapid A user can use a larger capsule or cassette module to speed up their process or scale down to reduce production costs.

In addition, not all molecules will progress from the clinical phase—where the demand is only a few batches—to the commercial stage. Sartobind® Rapid A can be used to offset the COGs during the clinical phase, limiting upfront investment until there is more certainty about drug approvals and the commercial market potential.

Conclusion

In the current biopharmaceutical landscape, contract development and manufacturing organizations, such as Nanogen, are often expected to run multi-product facilities. As a result, flexibility and low COGs are crucial to ensuring their competitiveness in the market. In this paper, we evaluate the performance of Sartobind® Rapid A with recommended protocols against resin-based processes. Overall productivity increased up to 16-fold while maintaining the expected impurity removal, contributing to significant cost savings. These findings demonstrate that Sartobind® Rapid A can be a more productive alternative to traditional Protein A resin chromatography for purifying mAbs.

Sartobind® Rapid A is part of Sartorius' wider process intensification offering, which includes a range of solutions to debottleneck manufacturing processes. Please reach out to your regional applications specialist to learn more about our Sartobind® Rapid A or our other products.

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